Race, Richard 2004

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Dr. Richard Race Interview

Office of NIH History Oral History Program

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Interviewee: Dr. Richard Race

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Maya Ponte: If you could first tell me about your background. How did you decide to become a veterinarian? Where did you go to vet school? And how did you end up at Rocky Mountain Laboratory?

Richard Race: Well, I guess very early I just realized I liked being around animals so I figured being a veterinarian would be a logical thing to do. I wanted to be my own boss, and I wanted to be outside, so that's the reason. As it turned out, I'm not my own boss, and I'm not outside. But I am around animals, so that part worked out.

I went to Colorado State University and my major varied depending on how my grades were going. I tried several majors from education to zoology. Veterinary medicine kind of just slipped in and I ended up getting into vet school so I decided to do that. Then I was lucky enough to get summer jobs working for the Public Health Service. So I was exposed to a lot of different situations that way. I worked for the Division of Air Pollution for a year and I worked a couple of years with some animal units in Bethesda- animal management type things. I liked all of those assignments and so I thought I'd like to work for the Health Service so I put in an application to coincide with when I graduated.

Scientists in Bethesda, where I worked before, told me that they had a job for me. So I thought all of that was pretty well set. In the Fall Richard Nixon was elected president and he eliminated all positions that were not physically filled. That meant that if the person wasn't actually physically on the job, that job disappeared. So when that happened, that job and all of those plans disappeared with them. They said they would keep a few applications on file and mine was one they would keep if I wanted to leave it there. So I told them to keep it active. That fall a job opened up here in Hamilton with Bill Hadlow, and since Bill was working with Aleutian disease in mink, he looked at my application and saw that I had 10 years of experience working on a mink ranch.

MP: When did you work on a mink ranch?

RR: I worked on a mink ranch starting when I was in about the 6th grade. I watered the mink and, as summers progressed, I did more and more important things on the ranch. I ended up working for the guy clear into my first / second year in college before I finally left him.

MP: And where was this? Where did you grow up?

RR: The mink ranch was east of Denver. It was in a suburb of Denver that is now all high-rise apartments, but at the time it was kind of on the outskirts of Denver. He'd pick me up in the morning, about five in the morning, and I would go to work and he'd give me a ride home about five in the afternoon. I'm sure with child labor laws nowadays there's no way I could have done that. But you know I had fun doing that, and it was my mink experience that really got me in the door here because Bill wanted that sort of experience. I worked for Bill primarily as a veterinarian doing a lot of pathology, doing a lot of necropsies, doing all of the clinical evaluations. We had 10,000 mice so I did all the scrapie evaluations for those.

MP: Did you have experience doing necropsies before you came here?

RR: I had some based on the usual veterinary curriculum, doing a fair amount of pathology, but it's all dogs, cats, horses, cows, but not mink. I' d never done postmortems on mink, but I learned. I developed a greater appreciation for what could really be learned from pathology when I came here where the animals that we were looking at were animals that you were able to put down that were really sick. The tissues were fresh compared to the diagnostic lab in vet school where the animals may have been dead for days. I didn't have a real appreciation for it, but when I got here and I actually got to examine fresh specimens, it was a whole different kind of thing. It was really interesting. Everything was different. So I really liked that. I did that kind of work with Bill for about 10 years until the laboratory was reorganized. That was around 1979. Basically what was done is that Bill Hadlow's resources were reduced and Bruce Chesebro's were increased.

MP: And why was that?

RR: People in Bethesda in charge of laboratory direction wanted to emphasize different aspects of the research problems - more molecular and less practical type things. They offered me a choice of either staying with Bill or going with Bruce, but strongly encouraged me to go with Bruce. That was okay with Bruce, so that's what I did.

MP: Did you know Bruce very well at that time?

RR: Not really. We'd worked within the lab a little bit because of collaborations between Bruce and Bill Hadlow, so I was involved in many of the experiments if they involved the animals, but I didn't know him real, real well. When I went to work with Bruce one of the first things he told me is that he didn't want me doing strictly work with animals. He said he "didn't need someone out there just taking care of animals. You're going to have to do a little bit of research." That sounded okay. So I started doing that.

MP: What did you think at the time? Like how much did you know about what was going on in the laboratory?

RR: Well I knew quite a bit already because I had been working with people in the laboratory all that time, plus I had done some experiments of my own. But now it was more like he was telling me you can have 100% of your time to do science, you don't have to do the animal stuff. And so that was a big change, but I found interesting problems to pursue. That's the last 25 years –

MP: So what did you start with when you came to the laboratory?

RR: In '79 we started to phase out a lot of what Bill had been doing, so the scrapie work really decreased to essentially nothing in terms of what anybody was doing with it at RML. Marshall Bloom was working on Aleutian disease and I had been working with Bill on AD so I continued working with Marshall on Aleutian disease instead of with Bill. My first independent projects involved studies aimed at defining antigen specific immune responses to Aleutian disease, viral antigens.

MP: So you started out looking at antigen / antibody interactions in Aleutian disease.

RR: It was an immunology / virology kind of a combination and then, I think it was in 1983, Bruce and I were talking and Bruce said, "The scrapie problem, is just too interesting to leave. We need to figure out something to do to rejuvenate the scrapie project." So Bruce organized and we had a world-wide conference here inviting everybody we could think of that was prominent in the scrapie field to give seminars.

MP: When was the conference?

RR: I think it was close to '83. I'm not sure of the exact year, but the idea was to get all of these people together, figure out what the people in the field were doing and then try to decide for ourselves what we could do, and what ideas we could get from them that we could pursue. We did that, and I don't know if I really got the idea from there, but I decided to look at cell culture models again, because the animal models are expensive, tedious, and they take forever. So one of the first things I did was actually try to develop a cell culture model for scrapie based on the Chandler scrapie model. I analyzed at least 20 different cell lines including every neuron based cell line or brain derived cell line that I could get my hands on. I tried to infect them and obtained one line that I was able to infect.

MP: And did you have to do anything special? I'm just curious because I know people had been trying to infect cells for a long time. Were there any special techniques that you used to optimize or how did you manage to do it?

RR: Well, the way we found it where I think other people hadn't is that the key was to do limiting dilution cloning of our cultures. We would overlay a culture of cells with a homogenate of infected mouse brain. At this point most people would have taken the culture and would have followed it through several passages. What they found – always found – was the primary culture would have infectivity but after a couple of passages the infectivity would be gone. What we did that was different was to limiting dilution clone the primary culture that we infected. That means we broke it up into single cells, grew each of those to a large number, and analyzed them for infectivity by inoculating mice, which was the only assay system available at the time. I think initially we had about 200 clones. This is a huge amount of work in terms of the number of cultures and mice and time. From all of the cell lines we tried to infect only one was infectable, one derived from a mouse brain tumor.

MP: How many mice did you use?

RR: We inoculated 10 mice for each one. The parent line was frozen in liquid nitrogen for later recovery. Then we just waited. We didn't think anything would happen, but about 150 days later the mice from one of the clones were sick with a scrapie-like syndrome. And then I think one other did also, but after longer incubation periods. So we ended up with 1 or 2 positives out of the 200 we screened. We then began passing the cells in vitro again from the positive clone and did additional limiting dilution cloning at several times until we had a cultures with nearly 100% of the cells infected. Now we had cultures that were really useful. When PrPres was finally discovered we were able to use PrPres as a marker for the infected cells which made the cells really useful and decreased greatly the need for mice.

MP: Much easier than having to screen it every time?

RR: Yes, and now these cells were infected at a level where biochemical analyses were feasible. People all over the world are now using these cells or have modified them. They've refined them and modified them and stabilized them, growing them in different kinds of media. They've manipulated them in different ways.

MP: Initially, did you share some of these clones with other labs or did they just create their own based on your techniques?

RR: Some created their own based on our techniques, but we shared with a lot of people, realizing that this wasn't the simplest thing to do. Anybody that wanted the cells could obtain them from us, with the usual transfer agreements. One thing that is kind of interesting is that Stan Prusiner will claim credit for developing the cells. We published the data a year before he published it, but he will always say, "Subsequently Race, et al., found the same thing." I can remember a meeting in Canada where I hadn't published this yet, but I presented the data and Stan was at the meeting sitting in the front row and he was writing. I had never seen a guy write so fast in my whole life. He was writing everything down that I said. Everything! That worried me enough that I came back and wrote up my results immediately. Our publication is a year ahead of his. So whatever he says, it isn't true and he's done that with a lot of people on a lot of topics.

MP: Did more people use your N2A cells or his?

RR: Ours, probably. The N2A cells are available from several sources including commercial outlets. Our infected cells have also been widely distributed around the world. Nowadays we just try to find the nearest lab. If somebody in Europe wants them, they can get them there. We still have people ask for them. They're still a little bit tricky because they're somewhat unpredictable in how long they will retain their infectivity. So with different people handling the cells in different ways sometimes you can get them to go hundreds of passes and not lose any infectivity. Sometimes you go three or four passes and it's gone. So they are kind of a touchy cell line. Some people have a good feel for them; you're looking through the scope and you're looking at cells. If you have experience with it and you look at – some people look at a cell and they think it looks fine, other people look at a cell and they can tell it's not quite right. It makes a difference with these cells. Basically, you have to have a feel for them. It's kind of like people and plants. You know people who talk to their plants; it's a bit like that.

I've had technicians that can grow these cells and have no problems with them and I've had other people that have all types of problems and I know it's just because they didn't have a good feel for them. They weren't looking at them often enough, or not being careful enough or would try to push them over a weekend. Just little things like that. Any of those things could result in a loss of infectivity. So the infected cell lines are still not the easiest cells to keep, but for people who pay attention they're pretty good and other investigators have standardized various parameters like kinds of media, fetal calf serum etc. that have helped reliability.

MP: I see so it's a little bit of a tricky thing. It requires certain skill on the level of the technician or the person growing the cells.

RR: Yes. It requires someone to continuously keep track of what's going on and to periodically monitor the cells to make sure that they're still producing at the level that they should be. If they aren't, then you need to either re-clone them or do something to get them back to where you want them. If a person wants to use cells they need to realize it is time consuming. They are going to have to spend some time at it.

MP: And as you were saying, no one previously had done limited dilution in order to select the cells. So what might have been happening previously is that the uninfected cells just grew over the infected cells?

RR: Right. Other people would get primary infection when they first put the infected material on the cells or brain cultures, would take homogenates from the cells and inoculate them into mice and find that they were infected, but whenever they passaged the cells they lost infectivity. Most of these attempts started with primary cultures rather than established but uninfected cell lines. Cloning was the key.

MP: Wow, so that was a critical insight to do that. So then you'd select single cells and get 200 clones, was this for each cell type you were looking at?

RR: No, we finally just used the neural 2A cells. I chose those because an investigator in Washington, David Kingsbury was working on scrapie questions for a couple of years and had used them with some success on other kinds of experiments. I thought they might be worth looking at. It turns out that these cells actually trace back to a mouse brain tumor. The cells were obtained by at least three different groups each of which re-named them. I obtained some from ATCC, some from an investigator at NIH and the neural 2As from Kingsbury. All three of these cell lines were infectable but no others that I checked so there is something really unique about them.

MP: The same original tumor from a mouse, right?

RR: Right.

MP: A single mouse tumor.

RR: The cell line had three different names depending on who had it but all from the same original tumor. All three of those could be infected and every other CNS line out there at the time wasn't, at least not in my hands. People now, like Sue Priola here, have managed to infect some fibroblast…

MP: Is that because she's using a slightly different technique?

RR: It's a similar technique but when we got the neural 2As working we didn't really look at this. We kind of quit looking at other cells and concentrated on using the ones at hand but she looked at other cells and then she started looking at different scrapie agents as well, besides just Chandler. She's infected cell lines with the 22L mouse agent and some of other cell lines. So I think we will get more and more lines.

MP: That's an incredible project to undertake in the laboratory, and you produced a tool that is now used all over.

RR: I remember talking to the board of scientific councilors about it and they said, "It's probably really a long shot, but this is probably the place to do it – government with hard money." He said, "It's probably not something you want to give a college post-doc to do but," he said, "you might as well try." So that worked out real well.

MP: That's great. That also shows why it is important to have intramural research to have a place that can do that kind of work, take that risk. And how long did that take?

RR: I would say probably two to three years, because just to get the mice to come down takes five months. I'd say it took - from the start of the project to publishing the first paper - probably three years.

MP: And so for the mouse bioassays those took about five months and you used about 2,000 mice to do that?

RR: I never counted them. I think we ended up analyzing 100 clones but had at least 100 more to do if needed.

MP: So about a thousand mice?

RR: Which here, in those days, wasn't many. When I started working for Bill Hadlow in the early '70s, we had 10,000 mice under experiment on any given day of the year.

MP: That's incredible. So this is when you were working on some of the pathogenesis work?

RR: Right. Trying to titer scrapie agents from sheep by inoculating dilutions into mice.

MP: So you examined thousands of mice with scrapie. You must have become very familiar with the signs. How many would you do in a single day?

RR: Well, I would basically look at every box of mice, and it was really a pretty easy diagnosis. You could go through the racks real fast. I looked at all 10,000 mice once a week.

MP: And what did you look for?

RR: Just looking for clinical signs. Most of the time with the sheep material going into mice they would become lethargic or somnolent, just not very active would be the first thing you'd see. Over time they'd either get really fat or get really skinny. But mostly you would notice lethargy, just not wanting to move around.

MP: Was there any reason you could identify why some mice got fat and some got skinny?

RR: I didn't really look at that, but Richard Rubenstein from Staten Island had isolated out some lines - I think he did it with hamsters actually – where obesity was a problem. He related the obesity to some metabolic changes based on the location of lesions in the brain and suggested the mice could be a model for diabetes and islet cell problems.

MP: Did you work on both the natural infection in the Suffolk sheep and the goats that were infected with the Chandler strain?

RR: We didn't do much with goats and scrapie while I was here. I don't know what Bill did with goats before I got here, but the goats here when I arrived were used for studies of progressive pneumonia and we also inoculated a bunch with brain derived from people that died of Creutzfeldt-Jakob disease.

MP: Okay. So the goat pathogenesis he had done before you?

RR: I think he did a lot of that when he was in Europe, in Compton. I think they were using primarily goats. We didn't do much goat scrapie here after I arrived.

MP: For the Suffolk sheep study you were getting samples from Mission, Texas, right?

RR: Right.

MP: And did you go down to Mission, Texas to collect.

RR: Bill always went down there. He took Dick Kennedy who was his chief technician. They would go collect the samples. I have been to Mission, Texas subsequently, but not while I was working with Bill. After that I was there a couple of times for facility evaluations and USDA consultation type things. We still have some tissues down there.

MP: You still do?

RR: Well we did, but not recently. They really have a hard time finding anything. They basically chucked everything into some freezers and then some of those Revcos [freezers] went down and they didn't know for how long, possibly days or weeks. So the reliability of the original Mission samples is not good, so we haven't gone there for anything lately.

MP: I see. That's too bad.

RR: Yes. Another unfortunate tendency in those days was to collect everything (tissues) and put it into formalin. Everything that didn't go into formalin was generally thrown away. They didn't freeze anything. So when we started our sheep studies in recent years, I couldn't get the tissues I wanted – you know after PrP was discovered you wanted frozen tissues. You didn't want stuff in formalin really. You wanted it all, but in formalin and some frozen.

MP: What's the problem with putting it in formalin?

RR: Formalin tissue is fine for histology and you can still do IHC, but you can't do any biochemistry on the samples. Formalin essentially ruins it for PrPres analysis, using immunoblot or biochemical analyses.

MP: Is it because it denatures it or what is it?

RR: It cross-links the proteins so they can't be retrieved. We've tried several ways to retrieve the tissues but we haven't found a satisfactory way to do it yet. Bill Hadlow collected tissues from dozens of sheep at Mission over the years. He would freeze just a tiny, tiny bit, like maybe 100 mg equivalents of tissue. The rest would all go into formalin or be thrown away. So we've got just dozens and dozens of animals that would be a huge valuable resource even now, even today, if there had been enough frozen tissue available. Nobody was even thinking that way then. When we did initial studies to show that immunoblotting was a better technique than microscopic evaluation, I couldn't get the frozen tissues, so I worked with people at USDA in Ames and they found some field veterinarians that tracked some highly infected flocks for us and actually collected tissues specially for us and froze plenty so that I could do several studies. Nobody else was collecting anything frozen. Nobody was anticipating that the frozen tissue might be valuable.

MP: Because until PrPres was discovered and IHC became a potential technique to use…

RR: There was no reason. With IHC, formalin doesn't interfere. That's fine for IHC. So you can still make the diagnosis, but if you wanted to do some kinds of biochemistry on the samples you're out of luck. You couldn't look at the glycoform-patterns, have tissue for bioassays or other analyses of the proteins. You couldn't look at the PK resistance – all those kinds of things couldn't be done and are now part of the basis for distinguishing TSE strains. So it's important to get tissue both ways - some in formalin and some frozen.

MP: Okay let's go back. So you did the N2A studies, using limiting dilution, you cloned these cells and then, once you had those cells, what did you decide to do next?

RR: Well, we tried to characterize the cells a little bit better. We looked at the frequency of infection in the cells. How long could the infectivity last? When PrPres came along, we determined how many cells were needed for a PrPres signal. We showed that PrPres could be used to track what's going on in the cells and not too long after that Byron Caughey and his people started using the cells pretty extensively. At about this time I decreased my use of the cells. I moved on to other things. I was getting tired of being a slave to all of the cells we had growing.

MP: Then what did you move on to?

RR: What was next? PrPres had been discovered around then, so I moved on to sheep scrapie diagnostic problems. Better diagnostics for TSE diseases is considered one of the most important TSE problems. Because we knew that PrPres was in brain and we knew we could detect it easily by immunoblot, I wanted to see if we could use immunoblotting of brain for diagnosis of sheep scrapie.

MP: And did you have sheep here or who did work with to get samples?

RR: I obtained samples from Al Jenny at USDA in Ames.

MP: So did you have a working relationship already with folks at the USDA or did you develop that relationship out of this?

RR: I just developed it. Nobody here had done anything with the USDA at all. I can't remember – I think they invited me to different meetings and evaluations so I got to know some of the people. Once I got to know them they were happy to send samples or help however they could. Initially they sent to me a bunch of samples representing scrapie infected sheep. We analyzed the brains using immunoblots and observed really strong, really beautiful PrPres patterns. I mean - just knock your eyes out, they were really nice. And so we obtained many more samples - unknowns - from USDA where we looked at them by immunoblot and USDA looked at them by microscopic anatomy, which is what they usually do. We were able, doing that, to come up with far more positives than they came up with. We had a lot of meetings over the years about the results, and basically proved that – we wrote papers on it – that immunoblotting was a much more sensitive technique than just looking under a microscope. It didn't require the huge amount of expertise that the microscopic evaluation required where Bill Hadlow and Bill Taylor and a few other pathologists were about the only ones in the world that could pick up these really early cases by looking through a microscope. With the immunoblot technique just about anybody could tell the positives from the negatives. In a few days we could train somebody to interpret the blots. It was definitely better. Everybody agreed that the technique was better. We had multiple meetings, and there would be four or five of us scientists that were sitting around talking about it –

MP: From the USDA and from NIH or from where?

RR: Mostly from universities and the NIH. I was the only NIH person, There were several from the USDA, mainly pathologists, who were the ones that were looking at all of these slides to diagnose scrapie that way and there were administrators. We'd all be sitting around talking about the differences in diagnostic methods and the pathologists, their pathologist, would be sitting at these meetings and saying to these people, "Immunoblotting is a better technique." I mean they would say, "This is much better than what we're doing, why don't we switch?" And then the big argument would be about who is going to do the work if it becomes more of a biochemical assay? And who is going to pay for it? And so it took them a long time to finally come around and work out the details but everyone was convinced. I think we really deserve the credit for initially pointing all of this out and proving the point.

MP: But why was it difficult to figure out who was going to do it if it was an easier technique?

RR: Well because USDA had all of their little compartments. Was this going to go to biochemistry or was this going to go to pathology? If we have to do it, we're going to need more people. Who is going to pay for it? Are you going to hire more people for us?

MP: Shifting of resources.

RR: Right, and then just very shortly after that Janice Miller wanted to do the immunohistochemistry on the samples. So she did the immunohistochemistry on the samples. We did a comparison where we did immunoblots and Janice did the immunohistochemistry. We analyzed a large number of sheep samples, positives and negatives, blinded. As I recall she found one that we didn't find and we found one she didn't find, out of a lot of samples. So it was obvious that immunohistochemistry was just as good as immunoblot and so the matter of which technique depends on what the goal is. If the goal is purely diagnosis, then use immunohistochemistry because one can process more samples in the same amount of time for less money. If you're doing biochemistry or you're doing something like that, then you use immunoblot. I think what the USDA does now is use immunohistochemistry as the first test and immunoblot where the immunohistochemistry is questionable.

MP: So immunoblot is just running a western blot?

RR: Would you like to see one? Okay, so this is an immunoblot. These happen to be mice, but all of these bands are PrPres and that's all that's on here; this is antibody specific for that. So if you see banding patterns like this, or they can be different depending on the species and a lot of other variables - that's positive. There can be subtle differences for different strains or tissues or species but this gives a general pattern.

MP: So you have the pattern - unglycosylated, mono and diglycosylated. And the antibody should only bind to PrP anyway?

RR: Right. And you can do controls.

MP: Did you say it's faster to do IHC than it is to do an immunoblot?

RR: I think it is possible to do more IHCs in a given amount of time than immunoblots because immunoblots are limited by how many samples we could run in the centrifuge and the fact that centrifuge rotors handle a limited number of samples.

MP: Companies like Prionics with their western blot, isn't that just a modified immunoblot?

RR: Yes, it's just a shortened immunoblot where they're not using as long of centrifugation steps or extensive tissue purification. They might miss an occasional animal because they're not centrifuging as long or purifying as much, but they get most of them.

MP: So it's a little bit shortened and they can run more samples at a time?

RR: Yes, because they can – with smaller volumes they can use rotors where they can put more samples in. So where we're running maybe 8, they can be running 24. So then it's just a matter of gearing up to whatever you want. People claim that they can run 1,000 samples a week. Well, we can run 1,000 samples a week, too, if you want to give us enough people and enough centrifuges and enough equipment. It's just how much you want to spend to speed it up. I think Prionics advertises they can do it overnight. I think their whole centrifugation process is about 4 hours and then a quick blot and then that's it.

MP: How long is the centrifugation process for the immunoblots you do?

RR: I do a low-speed spin, a high-speed spin and another high-speed spin, so it takes me about a day. So even doing this long one with a higher level of purification, we still can do that in a day. Then the blot takes a day, so for us two days would be the quickest we could do it.

MP: So the USDA would collect samples from naturally infected scrapie sheep. This was all natural infection, not experimental?

RR: Right.

MP: And they were sending you samples, they were looking at some samples with their pathologists, and then eventually Janice Miller got involved, and she was also getting the same samples and doing IHC on them. To validate the different methods, what was required to be convincing?

RR: I think all of that was kind of taken together as convincing, and then they also, for the immunoblot, just the immunoblot part, they also took 25 samples – 10 normals and 15 positives - and they sent them to four different labs. They sent them here, did them themselves, they sent them to Dick Marsh's lab and I think to Rich Rubenstein's. So there were four of us that did the same 25 samples.

MP: And was it blinded?

RR: Yes it was blinded. We didn't know what was what. We got 15 of the positives and 10 of the negatives. We got them all right, all 25.

MP: How did they know which were positive and which were negative?

RR: They were positive based on microscopic examination. So these were ones that the pathologists had seen by light microscopy and said were positive or negative. The negative sheep came from a flock of sheep they keep that have been negative for decades. But they blinded the samples and sent them to us. We got them all right. The USDA guy was John Katz who was there at the time and I think he was very good. He only missed one and he was doing a much shorter protocol. He was doing more like what Prionics and those people would be doing today.

MP: Oh he was doing an immunoblot, too?

RR: Yes. All of these people did immunoblot. He got all but one and I'm sure if he had used the longer protocol and the higher purification he would have gotten them all. Richard Rubenstein only got like 12 or 13. He was using a little bit different technique and Richard Marsh got even fewer than that. He was using a dot blot technique.

MP: What's a dot blot exactly?

RR: It's kind of just a sloppy thing where he took an unpurified brain homogenate and put it on a piece of paper and then did the antibody reactions on the paper. And that didn't work very well. His result was not good compared to ours or Dr. Katz's.

MP: You were using the longer version and he using a slightly shorter one?

RR: Yes, for ours and John's.

MP: And so that was basically the validation trial?

RR: That's what I think they needed to convince them that it was okay.

MP: What did you do next?

RR: Probably the next emphasis relates to chronic wasting disease of deer and elk. Chronic Wasting disease was just beginning to be discussed seriously, though we had been watching it for a few years already as a potential problem. To digress, we also tried to be involved in BSE really early on. It came in around 1986, and I was trying to take samples immediately. I talked to people in Europe because we had all the immunoblot techniques worked out and we could have taken samples right along with them and done comparisons and so forth. I tried to get BSE tissues from them. I talked to people in Europe and they were always really friendly, "Oh yeah, we'll send those to you, no problem. We'll send those right to you," and the only thing they ever sent were spleens; they never sent any brains. They sent me spleens, but no brains. This was '86, right at the start of the whole thing. So we were there, but we just didn't get any cooperation at all, although they were really, really friendly when you talked to them. So they sent the spleens. I know in retrospect the reason they sent the spleens, but not the brains, is that they didn't care about peripheral tissues. They weren't worried about lymph nodes or lymphoid tissues. They were only worried about brains. So I think they figured, "Ah, send them the spleens." So they did. The samples weren't very good – I can still remember them, they were kind of watery. I didn't know what they did to them, but I didn't think they were very good. But I analyzed them anyway.

MP: They were sent to you frozen?

RR: Yes, they were sent frozen.

MP: But you didn't know exactly how they were collected or stored?

RR: That's right

MP: Did it look like there had been some autolysis?

RR: No. They just weren't very solid. They looked like they were watery. I just didn't like them, but I ran them anyway. They were all negative so I figured it was probably the character of the samples was just bad. In retrospect, now we know that they don't find anything in peripheral tissues so the negative answer was probably okay, but they never sent brains. I always tried to get them and I never got them. I have talked since, over the years, with other people who had exactly the same problem. They also were never able to get anything useful.

I think the Europeans figured, “Oh this is a neat problem. We’ll work on it. We’re not going to let anybody else have this stuff.” So that was kind of disappointing. We watched the BSE part of everything in the early days from the sidelines. Then chronic wasting disease came along – that was really of interest to us – I’d say probably the last three or four years I’ve worked primarily on chronic wasting disease, using immunoblots to look at different protein profiles, and then also trying to look at cross-species transmission to see if we can determine from an immunoblot which species of animal is infected and what the agent is. If you give us a sample, and don’t tell us what it is, can we figure out whether it is chronic wasting disease versus scrapie versus BSE? Can we figure out which of those agents had actually infected this animal? By immunoblot we weren’t able to that.

MP: There’s too much variability, or what?

RR: Yes, the range of variability within fairly large populations of deer and elk, and sheep, is just too great. We could tell BSE infected cattle from scrapie infected cattle; we got a really good separation on those. We could tell those apart, but we couldn’t tell any of the others apart.

MP: So with the scrapie infected cattle, was there variability in those immunoblots?

RR: They were pretty consistent, but what was interesting was that there was virtually no microscopic changes. So the pathologists that looked at the samples said that, “if we didn’t know that they had been inoculated, we couldn’t say they were positive.” There were some subtle changes, but they just weren’t convincing. When we looked at them by immunoblot they were just knock-out signals. I mean just really, really strong signals.

MP: In what regions? Were they different regions of the brain than were affected in BSE from scrapie-affected cattle?

RR: We didn’t look very carefully because the scrapie infected cattle didn’t have lesions. The PrPres was everywhere pretty much most areas of the brain. If there is a weak area, it’s probably cerebellum.

MP: But you found it in the obex?

RR: We found it in the brain stem. We found it in the cerebrum. We found it even in cerebellum, but not as strong a signal. So they were really different in that respect. A second pass was done and gave pretty much the same answer. Still no convincing microscopic changes, but huge amounts of PrPres. There’s a lot of questions now days about how much of the damage you actually see in the brain is due to the PrPres and how much is due to other factors like cytokines or materials released by astrocytes or other support cells in the brain as too what is actually causing the lesions. If you look at these scrapie-infected cattle where there is virtually no histologic brain damage, but tons of PrPres, it really suggests that something other than PrPres is important. That’s what people are looking at now, trying to figure out how cells are damaged or killed. Over the years it’s just gotten more and more sophisticated and that’s kind of where we are now.

MP: Did you ever try to do bioassays of the cattle infected with scrapie?

RR: We didn’t do any bioassays in mice. It might have been useful but since the cattle were affected we probably wouldn’t gain much with a mouse model. I don’t think anybody else did either. Since the animals actually got sick we just didn’t do that.

MP: And so you also – so with the BSE, and even with the scrapie infected cattle, you were finding sort of reproducible patterns by immunoblot right? Is that correct?

RR: Yes

MP: And then – but when you were looking at CWD and scrapie you were finding a lot of variability.

RR: A lot more variation.

MP: And how do you interpret that? Like, what do you think is going on?

RR: Well we think it might just be a much more heterogeneous population. The deer and elk can be just more heterogeneous or maybe they're infected with multiple strains. Maybe like in scrapie where everything looks pretty much the same, maybe we're down to – well we know there were a lot of different sheep scrapie strains, but maybe the ones that caused BSE were a single predominant strain is what they kind of think. Maybe in the CWD situation, you still have multiple strains sorting out in those populations. We just really don't know. That's one possibility if you look at the immunoblots. The deer just seemed to have a lot more variation.

MP: And what would be the implications if there are different strains, if CWD is more of a scrapie-like situation rather than a BSE-like situation? One of the things I'm interested in is the relationship between science and of disease management policy. What are the things that need to be kept in mind?

RR: Well if there are legitimately different strains then you're worried that some are going to be really virulent and others not. Some of the strains may pass through easily from say, deer to deer, elk to elk, deer to elk. Some of the strains might cross species easily, where others won't. So if you have multiple strains, what if you have one in there that crosses into humans really easily, whereas most of them probably wouldn't? That is a main worry I think, with strains, is that you have to worry about – in a way you're treating not one disease, but you're treating multiple diseases based on the strain characteristics. Kind of like influenza changes all the time, so you have essentially multiple strains of influenza. Some are less virulent than others. Some are worse than others and so that might be true for other TSE agents like CWD as well .

MP: So it's a little bit of a warning in terms of maybe interpreting studies. If you have a study with BSE where you're looking at how it crosses over into different species, you might be able to apply that across the board to BSE whereas with CWD it might be more difficult.

RR: Yes because BSE is probably the one TSE where it seems to be a uniform strain, where it seems to act predictably. BSE gives the same kind of patterns regardless of what animal species was infected. You know it looks like one strain, but with these others it doesn't look that way. There's more variation. And so you have more things to worry about.

MP: And with CWD, are you also interested in modes of transmission?

RR: Yes, we are, and the problem is that we don't really have here the kind of facility and animal holding areas we need to do those studies. To look at modes of transmission, you really need a facility where you can have the actual animals onsite so you can do controlled experiments. Although I've tried to suggest that and I've pushed about as hard as I can, it doesn't appear that it will happen. I've suggested it as much as I can and nobody seems to be too interested in doing that. So facilities now that can do it are Ames and the diagnostics center in Wyoming with Beth Williams and Mike Miller at Colorado State and I think that Katherine O'Rourke at Washington State can house a few cervids as well. So those are the people that have the potential to work with the actual animal. All we can use are laboratory type animals, cell culture etc. We are trying to develop transgenic mice. That is mice that express the deer PrP protein and would behave as if they were a deer or an elk in a number of respects. We're in the process of making those. We are quite a way along but there are at least two other people that have already made them.

MP: Rich Rubenstein made some, right?

RR: Rich Rubenstein made some and Glen Telling now has some so the two of them already have them.

MP: And have they been validated?

RR: Pretty much. I think Rich Rubenstein's are the best and the furthest along and he has a lot of data on them already that he hasn't published. But his, if they're legitimate, look pretty good with very short incubation periods, maybe too short to be believable. We're trying to make others and it's okay to make them because essentially each founder can differ from all the others. Where the transgene positions on a chromosome, what chromosome, how many copies, all of these things can make a difference. So having a lot of them or having a lot of people try to make them is not a bad thing. It's really a good thing, because somebody may come up with some that are better than others. So we'll see how all of that sorts out. We're trying to make them so we will be able to have our deer-in-a-mouse, so too speak. They won't be as good as the deer themselves, but they will allow us to do a lot and when we need to go to deer hopefully we will be able to collaborate with the people who can hold deer.

MP: Right, so then you would have the model system and you would have the actual animal to go back to if you found something interesting. What would be the issues of interpretation in going from the model system to the larger animal?

RR: Well you never know, but you have to do it, because you don't know if what you found in a mouse is going to be the same as what you're going to find in an elk or the deer. So work it out in the mice and then go to the real animal. That's pretty much what you have to do. So that's kind of where we are with CWD.

MP: Well, that's interesting. I did want to talk a little bit about the subclinical carrier. So how did you get the idea to put 263K into mice?

RR: Well, basically, the first observation was pretty much an accident; we inoculated a bunch of mice with 263K as the negative control on another experiment that we were doing. I can't even remember what the other one was now, but these were supposed to be the negative controls. At about 600 or 700 days post-inoculation, when we sacrificied the mice for the other experiment, we also euthanized the controls and did immunoblots on these guys. They were supposed to be negative but some of them weren't. We saw some PrPres in a couple of the mice, so we wondered what in the heck happened. We needed to figure it out. To do so, we did a more logical experiment and inoculated about 20 mice with 263K and then just started following them over time to see what would happen, and that's how that experiment evolved. That experiment took 6 years.

MP: The paper that was published in 1998, was that the one that you did those 20 mice for?

RR: No, I don't think that one was with 20 mice. I think that one was less. That was kind of where it started.

MP: That was less. I think I have it.

RR: I think it was the *Nature* paper right?

MP: Yes, the *Nature* paper. So that was just those two?

RR: That was showing the original observation, and the influence of the PrP gene.

MP: Okay here it is. So this was using –

RR: So that does show those guys. Okay, so that must have been first pass there. That's genotype. See, we were looking here at the influence of the genotype to see whether mice that didn't have PrP could become infected. And it was dependent on PrP. The few positive animals at the long incubation periods we interpreted as residual inoculum. That's a different issue than the persistent mice where we showed an increase in titer over time.

MP: Okay, because it is all the way out to…

RR: 294 days.

MP: And you still think there was left over inoculum?

RR: Yes because only 2 out of 12, 4 out of 12 died in those groups. And the fact that the incubation periods are so long means that there's not much infectivity present.

MP: And, what did you think when you first saw those?

RR: Well, I can't remember what we thought. We just thought something weird was going on, and did we make a mistake of some kind or was this really real? So then we continued to pursue it, just kept passing from mouse to mouse. The longer we passed them, the more efficient infection became. We followed several donors which was an important thing to have done, because by following those multiple numbers of donors we observed all sorts of variations in the end. Where some were hamster-tropic, some were mouse-tropic, some would go either way, and others lost infectivity all together.

MP: So you were somehow selecting for, or producing, multiple strains?

RR: The recipients, the individual recipients, were selecting something out of the inoculum, which might have been mixed. They were selecting something that just was compatible with their PrP type.

MP: So even something like 263K, which we think of as being a single strain, might involve multiple strains, or it might be adapting?

RR: It's adapting, we think.

MP: You think it's adapting?

RR: It probably started as pretty much a uniform strain, but it's adapting to the mice over time based on characteristics of both the inoculum strain and mouse breed. It doesn't happen quickly; it took years. Basically, it took the life spans of the animals for it to actually happen, but once it adapts – the other thing is in one of the later papers it shows how a lot of times the incubation periods of the initial passage, if the infection takes, will be very, very long. The second passage, same species, it's shorter and shorter and we usually think that you keep doing that until it stabilizes and when it stabilizes at a certain length that's probably, now a more homogenous strain. As long as the incubation periods are changing, the adaptation process is still happening between the host and what you gave it. So some of those, even at the time that we published that last paper are still not uniform, predictable strains but at some point, if passaged enough, would become stable, we think.

MP: The one with four passages?

RR: Yes, some of those really haven't stabilized yet. They're still changing.

MP: From generation to generation?

RR: They're still changing, but we didn't do another pass. We figured we had made the point.

MP: So that's it for this experiment?

RR: So that's it for this experiment.

MP: To you, what are the most important points that came out of this?

RR: Well, I think what it says is that in cross-species transmissions the original infection can happen but may take a very long time or even multiple passages in the new host species. These early events are not recognized because no clinical disease is observed. We may never know it is happening, but given the opportunity to be passed again to the same species, at some point it will probably become compatible enough with the new host where it will eventually cause clinical disease. So the initial infections may never be appreciated -you may never even know they happened. Cross species adaptation doesn't need to be an immediate thing. It can take multiple passages to happen, but it is possible.

MP: One of the things that you guys point out in the article which is, of course, very meaningful for disease management policy is with the recycling of animal protein from cows to sheep to pigs – that it's possible another strain could be developing, or adapting, under the radar, even though we haven't seen it yet.

RR: And people are real, real worried about that. I don't know if it is from our papers, but I suspect they have had a lot to do with the current concern. The USDA now has even eliminated chicken scratch from feed.

MP: Do you ever talk to people in the USDA about how they receive this kind of work?

RR: They don't like to hear things that complicate their lives.

MP: Who does?

RR: Yes. The implications for the changes they have to make are just huge and nobody thinks, on a really practical basis, that there is a problem, because you'd have to have probably everything worked out just right to have something like this happen in the real world. I mean to transmit between swine – well, even say deer and cattle – number one they'd have to be compatible enough, their PrPs, to where transmission would occur, and Byron's work with cell free reactions say that combination is unlikely. It could happen, but it's probably not a real strong likelihood. So then you're going to have to get two species together, concentrated, in an area where it is going to be able to infect the other. You're going to have to have a huge dose. How are they going to get a huge dose? You start thinking of all these things –

MP: Do they have to have a huge dose? So in the cases you reported, was this a large dose?

RR: Yes, at least in our experiments we gave them a lot. So they're probably going to need a lot in practical situations as well, and it probably isn't going to happen. The USDA doesn't like to hear things that complicate their lives, but Sue is on the FDA Advisory Committee. The FDA probably is a little bit tougher on things than USDA, because they're kind of an agency that can say well, you have to do this, but they themselves don't have to suffer any repercussion. It is USDA that would have to implement and make it happen.

MP: I wanted to ask you about intraventricular treatment. I noticed your name was on a paper with Doh-ura.

RR: Oh yes. Basically, what they do is insert a canula, – it's just a little canula in the area of the brain that you want, and then that's attached to a little reservoir of drug and so the advantage of that is that you can actually bathe the brain in these anti-scrapie type drugs. The problem with most of these is if you give them by the usual route, oral or intraperitoneal, most of them don't cross the blood brain barrier. So if infection is already into the brain most of these drugs are not going to work. The advantage of the cannula is that you bathe the brain with the drug and hopefully have some effect.

MP: What was your role in that study?

RR: I provided them primarily with the mice and the information about infection and characteristics of the mice, how to type them, etc.

MP: So they did the study back in Japan, but you provided them with the mice?

RR: We sent the breeding stock so they could get started with those mice.

MP: And do you know Doh-ura? Was he here for a while?

RR: Yes, he was here for a while and had done several studies. There are a lot of people here now doing drug discovery. A big thing in TSE research right now is drug discovery. Most of the major labs around the world have at least some effort in this regard. Dave Kocisko here does drug discovery full time. That's all he does now. He's gone through thousands of drugs and has found some pretty promising drugs. He will probably test several of these with the intraventricular cannulas.

MP: To see if things that might not cross the blood brain barrier could be promising?

RR: Yes. Dave and I went to Utah State to visit a guy they collaborate with to learn the technique, so I think when they are ready we can do the work here as well.

MP: So at some point you guys might do some intraventricular studies here?

RR: I think he will.

MP: Because I know they've already started – I mean, I'm sure you're aware in the U.K. that there are some patients receiving pentosane polysulfate with the intraventricular treatment.

RR: There are people that will try anything that you have. I mean, they are desperate. If you have anything at all that you want to try, fine. And so the idea is to find some things that might actually work.

MP: Do you think that there is really a potential to treat this disease, I mean given what you know about it, like the pathological changes and things that happen? What's your view of the possibility?

RR: I think there's a potential to arrest it at whatever stage you catch it, but I don't think that you'll be able to reverse the changes because amyloids are notoriously resilient to dissolving or eliminating. So I think if you had a person that you caught early, you might have a chance.

MP: But here's the catch, and this is the thing that's been boggling my mind lately - so these amyloids are difficult to eliminate once they're in a certain system an organ system, right? So why is it that in some of Hadlow's early pathogenesis studies that you do see the presence of the infectious agent in certain tissues that it then disappears from later in the course of disease, like lymphoreticular system in goats I believe?

RR: It doesn't really disappear; it just becomes a lower titer. The other thing is that in a lot of tissue…

MP: Or here, let me give you another one. In the BSE the infectivity studies that they've done in Britain where they show infectivity appearing in the intestine early on, and then it goes away from the intestine, and then they don't show anything coming back in the intestine until much later in the course of disease, what's happening?

RR: The amount that they're seeing is very, very small where they find PrPres in the cecum. The amount that they're seeing is so little that it's like – it's almost meaningless the amount they find. So that's probably why. But the tissue distribution in different species is really, really different. So that is an interesting topic because in BSE you find a little bit in the cecum and you don't find much anywhere else except the brain and spinal cord. In people that are infected with BSE, it is much more widely distributed. Then you see more in the cecum. You see tonsil and you see other lymphoid tissues affected. So when you cross the species barrier all of a sudden the distribution becomes a lot different, and if you look at sheep, it is widespread in sheep all over the place, many of the lymphoid tissues. In some animals, it is rare in lymphoid tissues, like elk or cattle. In some species, you only see it late in peripheral tissues, as if when the blood brain barrier breaks, when the animals are near terminal, it gets distributed into multiple other places. You get deer and elk where you find it in lymphoid tissues in deer widespread, but not in elk. So elk look like BSE and deer look like sheep. There are just a lot of differences. The thing about CWD is that the involvement of lymphoid tissues looks completely different in deer than it does in elk.

MP: Why do you think it might have come from sheep scrapie?

RR: Well, you'll get me in trouble. So the first CWD was seen in the Colorado/Wyoming area where Beth Williams started working on looking at it. So at Colorado State, at about that time in the '60s, several people were working on deer, primarily dietary studies and hormone studies looking at antler growth, things like that. So they had deer there pretty much all the time that people were working on. They freely admit that they'd bring deer into the facility and a lot of times they were interested in looking at primarily the babies and how changes occur in them as they develop. So they would bring the adult deer in, wild caught deer a lot of times, let them have their babies, and then they'd turn the adults loose. They'd turn the mothers back loose into the wild. At the same time, I am told, there were people working on sheep down there, and the controversy is - were they working on sheep scrapie or not? I know a guy, a veterinarian, that says that he worked there in the '60s, late '60s, as a vet student, and he knows for a fact that there were deer, and sheep with scrapie, not in the same pens at the same time, but that at different times there would be a sheep with scrapie there or there might be deer there. And so what might have happened is they might have actually infected them there and then turned them loose. So how did they get spread around the country? There's a lot of activity that goes on with deer and game farm type situations, some legal, some not, but the bottom line is that the animals get moved all around the country and if some happen to have CWD and then escape, which is a common occurrence, then the disease could have been spread that way. So that might be where it came from and got distributed, but you can't prove it. You can only speculate.

MP: Maybe. Who knows. But there's a potential?

RR: There is a potential. Is it just coincidence that it happened at CSU where they happen to be working with deer and elk? We will know better when they get a better idea of where CWD is in the country, if it is more widespread than what we think.

MP: Right. As they start improving surveillance.

RR: Right as they start improving surveillance. They're supposedly looking at least some animals from every state this year. So if it turns out that it is in all of those places, and then I don't know, then maybe it gets back to the prion only guys, and the protein only guys' idea of spontaneous conversion in all those different places.

MP: So let's move to that. That's a good lead in for my next question, which is: do you use multiple models of the agent at any one time? Or how has your idea of what the agent might be evolved over the years?

RR: For me, it's just kind of looking in from the outside as to what it is. I don't do anything that actively tries to determine whether it is protein versus something else. I think that's a biochemical question . I think that I would be way out of my realm to try to do those kinds of things, but so far at least I think the protein idea certainly wins among most people. I think that there are a lot of good arguments to suggest on either side. I think even the protein only people believe that there may be other molecules involved. Whether it's a virus or not, who knows, but the protein only may not account for all that's seen. Prusiner supposedly now has a paper out that's supposed to strengthen the protein only theory by quite a bit, and I know Bruce and some of the people around here who have seen copies say that there are some problems with it. It seems to be moving now, but I think the people trying to prove the protein only theory outnumber the people that are trying to prove the viral type theory by about ten to one.

MP: Okay, well let me ask you this question, which is then a little bit more relevant to your work. What relevance does a model of the infectious agent have for the work that you do? When you are looking at immunoblots, looking at pathogenesis, infectivity - at what points in your research does it matter what model of the agent you use?

RR: Well, we kind of operate on the idea that it is what it is and whatever we learn about the protein and whatever we learn about the disease, is going to be valuable, regardless of what the agent is. I think what it is will have more relevance in terms of treatments and intervention type things, because I think there it can make a big difference. If you know you're dealing with a protein only, or if you find out that you're actually dealing with an infectious agent, I think how you approach that would be really, really different. So I think, from that point of view, that's an important point, but I think for at least learning about the diseases - what it is – you know we thought early on, when the argument all started, that this was going to be something that we had to know the answer to right away. I mean it was critical to know the answer to it.

MP: And why did it seem critical?

RR: Well, because of the way you would approach the two different – the way you would approach a viral infection versus a protein only thing. With the virus you ought to be able to develop a vaccine or you ought to have a lot of approaches, even the logical approaches that you wouldn't maybe have with a protein. So it seemed like it would be really, really important, but the way it's turned out is that everybody accepted that the protein is really important regardless of whether it is the agent or not, and so everyone's pretty much concentrating on that. I think everything has moved forward pretty well. If it is a protein, it behaves as an infectious agent, so we're okay that way. I think treatment, how to treat it – knowing would be important.

MP: Would be helpful, definitely.

RR: Yes, would be definitely helpful.

MP: Do you work with the New York Institute for Basic Research scientists very much?

RR: Mostly it has been trading reagents, antibodies and that sort of thing. Rich Rubenstein was always there in the early days with USDA, when we were trying – you know when we were pushing the immunoblot assay. Rich was always at those meetings. A lot of the consultations that I've been at with USDA in the late '80s / early '90s, Rich was a part of those. So it was usually Rich, myself, and Richard Marsh at Wisconsin, who died, and then USDA people.

MP: So Rich Rubenstein is the one that you know the most?

RR: I've known him pretty well.

MP: And then, in terms of reagents, have they been helpful with things like 3F4?

RR: Yes, the New York guys have always been great. I mean, they never hoarded anything. Any reagent that they had, if you had a use for it, they were ready to send it at any time. And what they sent you was what they said they would send you. There wasn't any – if they sent you a protocol, it was what they actually did. If they sent you a reagent, it was what they said it was. And there were never any delays. If you wanted an antibody from Rich and you called in, they gave it to you. I think they had Material Transfer Agreements at the end, but as soon as that was signed the stuff would be there the next week. So they've always been really good that way.

MP: What have been their critical contributions?

RR: I'd say mostly tools. They had antibodies; they had some clones of different TSEs and some good scrapie clones and things like that. So they were kind of technocrats in a way.

MP: So they maybe helped in some ways, maybe helped further the field just by developing these tools and spreading them around?

RR: Sure, and making them readily available. I'm sure that if our transgenic mice don't work out, I'm sure Rich would send us his.

MP: And what about future directions?

RR: Well, for me, I'm going to retire.

MP: Are you really? When are you retiring?

RR: In about one year from now.

MP: Oh, you looked at the clock didn't you?

RR: In about ten minutes. No, I figure next August I'm going to retire.

MP: Okay, so you have a year left and will you continue focusing on CWD until then?

RR: CWD and all of these - I'm just drowning in transgenic mice of all kinds. We've got 11 different transgenic mouse models of – actually we have more than that because each of these deer guys is separate. So right now I've got about 21 different transgenic mice that I'm trying to manage.

MP: Included in that is also the mice that are expressing tissue specific PrP, the hamster, the knockout mice of the hamster tissue specific PrP? Those are really interesting models.

RR: Those have been useful. A lot of people have used those and request them from time to time.

MP: Within this country or in other countries?

RR: All over.

MP: All over the world?

MP: All over the world basically. So basically that's where I am. My biggest headache right now is just trying to sort through these mice and analyze them, figure out which ones are okay, which ones aren't, try to get them to a stable point where we can turn them over to the breeding colonies and not have to worry about them. So I'm doing that, and then the CWD work. I've got a whole lot of CWD experiments underway and following the monkeys. We have quite a few monkeys inoculated with CWD. If they get sick before next August I would likely stay longer.

MP: How are the primates doing?

RR: They're just happy.

MP: They're happy, no sign of clinical disease yet?

RR: No.

MP: And what do you think have been the most interesting things from this lab at least, data to come out of these transgenic, the tissue specific transgenic models?

RR: They're all pretty interesting, I think. Well I think what has happened is with looking at the neuron specific versus the astrocyte specific is that – it gets real confusing. You know, this argument has gone on for years, about whether neurons were really important or astrocytes were really important or what is going on and there were people on the neuron side and people on the astrocyte side, and the bottom line is that these cells probably all talk to each other in different ways and they all produce different chemicals and different compounds that cause various kinds of interactions. So where all of that is going, I think, is leading to a better understanding of how different cell types interact to cause damage to cells.

MP: This is where it is astrocytes only expressing PrP?

RR: Right. And with all the really neat electron microscopy work it looks as if what astrocytes are producing is actually damaging neurons, even neurons that don't have PrP in them. It looks as if maybe the astrocytic response to infection may actually be the most damaging.

MP: But it's only damaging nearby neurons, is that correct?

RR: Yes, it's not necessarily in them. It's just in the area of them, this so called diffusible PrP, which is kind of a nebulous term, too. I think those papers have really lead to a better understanding of what's been going on at the more molecular level within the brain and that's really important in the long run because if you can figure out how cells were being damaged maybe you could figure out how to block that. So that might be another way that would tell you what sorts of drugs maybe to try, because if you knew the particular cytokine produced by astrocytes is what is actually causing all the damage, then maybe you could target that point. Different people are looking for different sites to target to prevent infection. Like with bacterial infections, or whatever, if you can just block one reaction, even if you have a hundred step molecular reactions to finally activate some virus, you only need to block it at one place. So it's the same sort of idea. The goal is to try to get as much basic information as possible to allow us to determine where to target therapy or prevention.

MP: That makes a lot of sense. Well those are all the questions that I think I came up with for you. Is there anything else that you can think of that we haven't talked about?

RR: I guess if you think of something else you can call. You hit most of the main issues I think. The big thing is the reason why people care about TSEs. These diseases used to be just agricultural problems. They still are agricultural problems, but people didn't care about agricultural problems. They'd feel sorry for the farmers, the fact that they can't make money and their animals die but, you know, it was the variant CJD which changed everything. I mean, I doubt if you'd be here wanting to know anything about this if people were not dying and people were not really worried about the cross-species transmission and can you get it or not. And if they can get it, they're going to be really worried, and if they can't, it is going to revert back to an agricultural situation. So that's where we are with CWD. As soon as people know the answer to that, they'll either be really, really excited or they'll forget about it again.

MP: Is most CWD funding right now from agricultural research funding programs or from human health funding programs?

RR: It's kind of weird. It's kind of human health and then the Department of Defense has actually provided huge amounts of money here lately for –

MP: Is that what's funding the CWD primate study?

RR: Not here because we have hard money, but there are a lot of people in the outside world that will be funded with DoD money. There are a lot. I've gone to a couple of meetings where the meeting itself was funded by DoD money, so that's been a source. There's definitely more money available than there used to be. Most of the money – what they're really desperately looking for are diagnostic tests or tests where they can – they need to be able to diagnosis this before animals get clinically sick and quite awhile before, because if you look at an animal like a sheep with a three year incubation period, you want to be able to start picking those animals out by the time they're a year or a year and a half with a blood test or some natural excreted product, saliva, something that you can get really easily, and we've got – the diagnostic test that we have now are great. I mean, give us a piece of brain and we can make a diagnosis. I mean no problem. But that just isn't good enough. If you can get an early test so that you can start testing animals and pulling them out of the population, you wouldn't have these diseases. If we had an amyloid test for scrapie that you could apply a third of the way through the incubation period, there would be no scrapie. It is the fact that these animals get to a clinical state before you know that they're sick that is a huge, huge problem, because by the time they're sick, they've already had plenty opportunity to spread infection to all of their offspring from the last couple of years. And that's a big problem.

MP: So ante-mortem diagnostics is really a big focus?

RR: It's the big one right now. If somebody came up with a test like that they would be set for a long time and very rich, if they weren't with the government. I don't personally think such a test will be based on PrP. People have just beat PrP-based assays to death since that's all we currently have. I mean, everybody is looking at PrP because it's the only marker we have. There's probably some other antigen or some other protein, or some other change in metabolism of an animal when it becomes infected that somebody eventually will find and that will be the basis for the ante-mortem test. But that's looking for a needle in a haystack.

MP: Sure.

RR: It's like the viral I.D. of TSE disease. It's hard to find something that you can't identify. For example, if you have a molecular marker for what you're looking for, it's really simple. It's like the protein only argument. When they say they eliminate all the nucleic acid in an infectious sample, it's not really true. For example if you take a sample of the brain and you do DNAse treatments to eliminate all of the nucleic acids detectable by conventional methods, and then you look for DNA in those samples, there isn't any. But if you've seeded the original sample with an iodinated or some kind of radioactively labeled nucleic acid, do all of the purifications and DNase treatments, and then look for the DNA that was seeded using a molecular probe, they find a lot of the seeded DNA. So the idea that all of the nucleic acid was destroyed is false, and the preparations thought to be nucleic acid free aren't. Okay, but what DNA are you going to look for? Without a probe for what you're looking for, you're not going to find anything. So you've got all kinds of problems like that still.

MP: So there's a lot to be done in this field.

RR: Yes, and when you read a paper, there is always information left out. I mean, if you're trying to sell a particular idea, you don't go to extra efforts to try to help the contrary opinion. This is what Stan does all the time, and you don't blame him for it. If he's a protein only guy, why in the world is he going to tell you the caveats that argue against it? He isn't going to do that, or nobody would think he was right. I mean, they just don't do that. I reviewed a paper yesterday where they presented the data in such a way that they could hide any problems that might have been there. That happens all the time. And if the reviewers aren't sharp enough to figure it out, those papers get published and then they serve as the basis for other papers. Pretty soon, something that had very, very little scientific justification is accepted as the gospel by other people who haven't gone back to the originals, who haven't done the evaluations. They count on reviewers to do a good job. If they don't, you get a lot of junk out there.

MP: So once something has gone through the peer review process, there is very little scrutiny after that?

RR: Right. That has always been true with the PrP protein only ideas. You know, the general scientific community doesn't know enough about it to pick up the really fine details that are left out.

MP: So unless they go to people within the PrP field for a review…

RR: Yes. And most people are just kind of casual observers from the outside and they kind of look at it – they always used to like to come to the TSE meetings in the early days, when Stan was first doing the protein only stuff, I mean people really argued. It would really get heated up. It would really get going. So people would come to the meetings because they wanted to see what kind of fights would break out. They don't do that anymore. There are some undercurrent battles, but there's no public shouting anymore. Everybody sort of gets along.

MP: So what are the things that someone with a background in prion research would look for in a paper when they're reviewing it that might not be caught by someone who doesn't have a background in the field?

RR: It would be just like any other field. You would just need a reviewer that knows the literature well enough to know when something just isn't right. What kind of buffer did you use? How did you present the data? Did you use enough animals?

(Interviewer asks RR to explain how data can be presented in misleading ways)

RR: I could have made this look really good. Okay, you see, look at this – this is just one of the – you can read, I'll give you this – this is just one of the immunoblots where we're looking at the lower bands - varied lower band relative to upper band for different animals. Well, on this one we're trying to see if these break out so we can tell differences. And here, I was showing you, we can actually tell the cattle with scrapie from the cattle with BSE. See, there's no overlap in the range. None of these black guys end up here and none of these white guys end up here. And that's the problem with that paper I just showed you. I could have made this look really good, what if I just decide to use the bar plots and don't show you each individual animal? The averages make the result look very good, but when you see individual animals you see the outliers that cancel the authors' interpretation of the data.

MP: Right, if you decided those were outliers or that they were false. Like you were saying, if you presented it in a bar graph or something instead of actually showing data points, then you can lose some of that information. You wouldn't see that they overlap.

(Interviewer asks question about why Richard Kimberlin didn't observe the subclinical transmission of 263K to mice when he did his experiments)

RR: The reason he didn't find it, the persistence, is that he killed his mice too soon. Two things: he killed his mice too soon, at like 300 days, 304 days or something like that, and number two, he didn't have PrPres as a marker to follow. So if he had done that same experiment after 1986 or so, when PrPres was being used, he probably would have found it. He probably would have found the same thing we did.

MP: That's so interesting. So with that technique available, he probably would have seen that there was the potential – he probably would have then held the mice longer and done bioassays at a later point.

RR: You would think so.

End of transcript